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THE PROTECTIVE PROPERTIES OF ANTI-POLIOMYELITIC HORSE SERUM

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In previous experiments in a series of 53 monkeys we have demonstrated the protective, curative¹ and neutralizing² properties of anti-poliomyelitic horse serum for experimental poliomyelitis of monkeys. The striking beneficial results observed by Nuzum³ and by Rosenow⁴ in the treatment of human poliomyelitis with immune serum prepared by injections of the poliomyelitic coccus in animals demand a thorough and extensive trial of the serum.

It is obvious that to obtain the best results some convenient and satisfactory test of the potency of immune horse serum must be adopted to determine its fitness for use in human poliomyelitis. I have shown that such a test² may be made by an accurate estimation of the neutralizing properties of immune horse serum in vitro for the virus as determined by monkey inoculations.

Such a test, when properly controlled with normal horse serum, and also with immune serum obtained from both convalescent patients and monkeys which is known to possess neutralizing properties for virus, affords accurate information.

Recently, Amoss and Eberson⁵ devised a therapeutic protective test to which he subjected a sample of Rosenow's serum. Two monkeys treated by intraspinal injections of the serum developed poliomyelitis. They conclude that the Rosenow serum is devoid of protective power.

With the purpose of determining the value and limitations of such a protective test, and in addition, to determine the protective value of our serum, I have made this test in 3 different series of monkeys.

The test rests primarily on the observation of Flexner and Amoss that intraspinal injections of normal horse serum or monkey serum set up an aseptic inflammation of the meninges and choroid plexus

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¹ Nuzum, J. W., and Willy, R. G.: *Jour. Infect. Dis.*, 1918, 22, p. 258.

² Nuzum, J. W.: *Jour. Infect. Dis.*, 1918, 23, p. 301.

³ *Bull. Chicago Med. Soc.*, April 21, 1917; *Jour. Am. Med. Assn.*, 1917, 269, p. 1247.

⁴ *Jour. Infect. Dis.*, 1918, 22, p. 379.

⁵ *Jour. Exper. Med.*, 1918, 27, p. 309.

in monkeys thereby facilitating the passage of virus from the blood stream into the nervous tissues and thus inducing characteristic lesions and symptoms of poliomyelitis. They found that only one substance possessed the power, when injected intraspinally, to prevent the localization of the virus in the nervous system, namely, the serum of convalescent monkeys which have survived a poliomyelitic infection.

The actual test is carried out as follows: An active, fresh virus is obtained by inoculating a monkey intracerebrally with glycerolated virus in the usual manner. By virus, I mean 5% emulsions of the brain or spinal cord of paralyzed monkeys and not cultures of the poliomyelitic coccus. The virus utilized in all experiments recorded in this manuscript was originally obtained from Dr. N. E. Wayson, of the Public Health Service, Washington, D. C. It has now been propagated through a series of 25 monkeys and has reached the maximal degree of potency for all monkeys not immune. When prostrate, the monkey is etherized and the spinal cord and medulla removed aseptically and a 5% suspension of the nervous tissue in normal salt solution prepared, centrifuged and 50 c c of the clear supernatant fluid injected intravenously into monkeys. Control animals and monkeys treated with the serum to be tested, normal horse serum and convalescent monkey serum are injected similarly and tested at the same time. Daily intraspinal injections of the serums to be tested are made each day for 4 successive days. Following a rest of 2 days 3 additional intraspinal injections are given.

SERIES 1

Exper. 1.—Monkey 87. (Control.) Feb. 7, 1918: Intravenous injection of 50 c c of the fresh prepared 5% suspension of monkey virus which was previously passed through a Berkefeld filter.

March 22: Monkey has remained well.

Exper. 2.—Monkey 88. (Normal horse serum control.) Feb. 7, 1918, 3 p. m.: Intraspinal injection of 3 c c normal horse serum.

Feb. 8, 12 noon: Intravenous injection of 50 c c of a 5% Berkefeld filtrate suspension of virus. At 12:30 p. m.: intraspinal injection of 3 c c of normal horse serum.

Feb. 9: Intraspinal injection of 3 c c normal horse serum.

Feb. 10: Intraspinal injection of 3 c c normal horse serum.

Feb. 11: 3 c c normal horse serum intraspinally.

Feb. 12: 3 c c normal horse serum intraspinally.

Feb. 13: Fine tremor of head.

Feb. 15: Hind legs weak.

Feb. 16: Coarse tremor. Flaccid paralysis of both arms. Prostrate in cage at 6 p. m. Paralyzed in all extremities.

Feb. 17, 9 a. m.: Moribund. Respiratory embarrassment. Monkey died at 8 p. m. Typical microscopic lesions of poliomyelitis.

Exper. 3.—Monkey 89. (Immune serum.) Feb. 7, 1918, 3:30 p. m.: Given 3 cc of fresh immune horse serum intraspinally.

Feb. 8, 11:30 a. m.: Intravenous injection of 50 cc of 5% virus suspension previously Berkefelded as above. Three cc immune horse serum intraspinally.

Feb. 9: 3 cc immune horse serum intraspinally.

Feb. 10: 3 cc immune horse serum intraspinally.

Feb. 12: 3 cc immune horse serum intraspinally.

Feb. 13: Intraspinal injection of 3 cc immune horse serum.

Feb. 14: Intraspinal injection of 3 cc immune horse serum.

March 22: Monkey remains well. Has never exhibited any symptoms of poliomyelitis.

Exper. 4.—Monkey 90. (Pooled immune monkey serum.) Feb. 7, 1918, 4 p. m.: Monkey received intraspinally 3 cc of fresh pooled immune monkey serum obtained by cardiopuncture from 2 monkeys convalescing from poliomyelitis.

Feb. 8: Intravenous injection of 50 cc of a Berkefeld filtrate suspension of virus. At 12 noon: Intraspinal injection of 3 cc immune monkey serum.

Feb. 9: Intraspinal injection of 3 cc immune monkey serum.

Feb. 10: Intraspinal injection of 3 cc immune monkey serum repeated.

Feb. 12, 13, and 14: Monkey received an intraspinal injection of 3 cc of immune monkey serum.

The clinical course of this animal is very instructive.

Feb. 20: Monkey appears to drag the left leg.

Feb. 22: Tremor and excitability. Definite weakness of both legs. Ataxia.

Feb. 23: Flaccid paralysis of all 4 extremities. Back muscles paralyzed. Monkey died at 1 p. m.

Sections of the pons, medulla, cervical and lumbar levels of the cord and of the intervertebral ganglia revealed marked lesions characteristic of poliomyelitis.

In these experiments both the immune horse and immune monkey serums were obtained Feb. 7 and used fresh without preservative. The pooled immune monkey serums were obtained from 2 monkeys, both of whom had survived a recent poliomyelitic infection with varying degrees of residual paralysis of 3-5 weeks' duration. The Control Monkey 88 receiving normal horse serum intraspinally developed a flaccid paralysis 8 days after intravenous injection of virus and died on the 9th day with marked characteristic lesions of poliomyelitis.

Monkey 90 receiving pooled immune monkey serum exhibited a delay of 9 days over Control 88 in the onset of the symptoms and died of typical poliomyelitis on the 10th day following the injection of virus.

Finally it is significant that only Monkey 90 treated by intraspinal injections of antipoliomyelitic horse serum remains well today and has never exhibited any symptoms of poliomyelitis.

SERIES 2

For these experiments a paralyzed monkey in the 1st day of complete prostration was etherized and a 5% suspension of the cord and medulla was prepared, shaken and centrifuged and the clear supernatant fluid injected intravenously according to the method of Amoss.

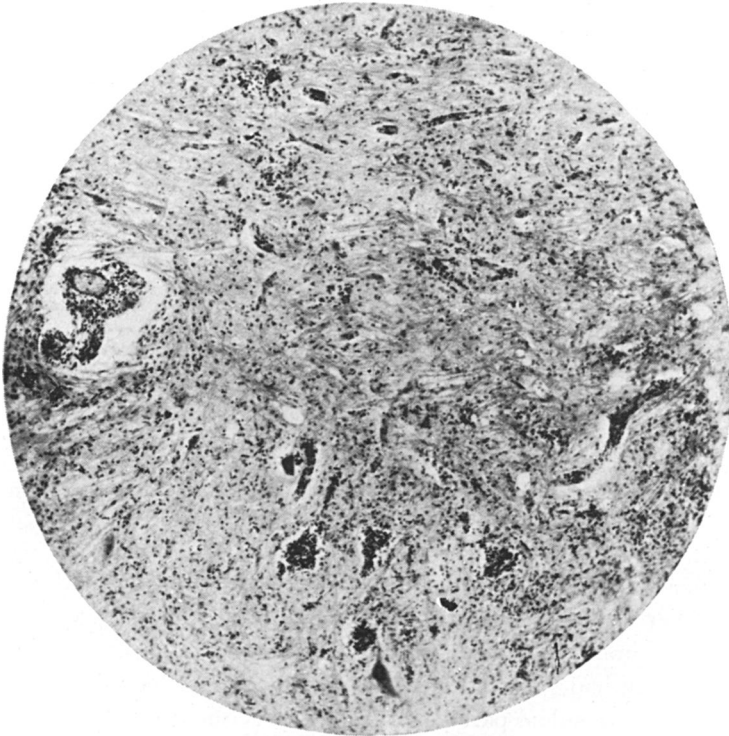


Fig. 1.—Monkey 93 (X70).—Typical neurophagocytosis, perivascular and interstitial lymphocytic infiltration in the anterior cervical horn of the spinal cord.

The immune horse serum was again obtained fresh the day of the experiments. The pooled immune monkey serum was obtained by cardiopuncture from 3 convalescent monkeys and divided into 2 equal lots. One lot was used fresh while the second was passed through a Berkefeld filter after previous preservation with 0.1% tricresol.

Exper. 5.—Monkey 91. (Control normal horse serum.) Feb. 18, 1918, 3:30 p. m.: Intraspinal injection of 3 cc of normal horse serum.

Feb. 19: Intravenous injection of 50 cc of a 5% suspension of virus. Followed by 3 cc normal horse serum intraspinally.

Feb. 20: Given 3 cc normal horse serum intraspinally.

Feb. 21: Intraspinal injection of 3 cc normal serum.

Feb. 22, 9 a. m.: Monkey has a complete flaccid paralysis of both arms and shoulders. Hind legs weak. P. m.: Prostrate in cage. Died at 8 p. m. same day.

Necropsy: Typical microscopic lesions of poliomyelitis in the brain and cord.

Exper. 6.—Monkey 92. (Pooled immune monkey serum.) Feb. 18, 1918, 5 p. m.: Intraspinal injection of 3 cc of fresh pooled immune monkey serum.

Feb. 19: Intravenous injection of 50 cc virus suspension. Followed by 3 cc immune monkey serum intraspinally.

Feb. 20: 3 cc immune monkey serum as above.

Feb. 21: Given 3 cc immune monkey serum.

Feb. 22: 3 cc immune monkey serum intraspinally.

March 22: Monkey remains well.

Exper. 7.—Monkey 93. (Immune monkey serum filtered and preserved with 0.1% tricresol.) Feb. 18, 1918, p. m.: Given 3 cc of above immune monkey serum intraspinally.

Feb. 19: Intravenous injections of 50 cc of virus suspension. Given 3 cc monkey serum intraspinally.

Feb. 20: Given 3 cc monkey serum intraspinally.

Feb. 21: Intraspinal injection repeated.

Feb. 22: Intraspinal injection repeated.

Feb. 23, a. m.: Generalized muscular weakness. P. m.: Paralyzed in all extremities and moribund. Died at 2 p. m.

Necropsy revealed marked lesions of poliomyelitis in the brain stem and lumbar cord.

Exper. 8.—Monkey 94. (Immune horse serum.) Feb. 18, 1918, 4 p. m.: Given an intravenous injection of 3 cc of fresh immune horse serum.

Feb. 19: Intravenous injection of 50 cc of fresh 5% virus suspension. Intraspinal injection of 3 cc immune horse serum.

Feb. 20: 3 cc immune horse serum intraspinally.

Feb. 21: 3 cc immune horse serum intraspinally.

Feb. 22: 3 cc immune horse serum intraspinally.

Feb. 28: Monkey remains well.

March 3: Left leg is weak. Monkey climbs awkwardly.

March 10: Monkey bright and active. Favors the left leg.

March 22: Monkey remains well. The muscles of the left leg are still weak. Prognosis as to ultimate complete recovery good.

An analysis of these experiments shows that the Control Monkey 91 treated with normal horse serum became completely paralyzed the 4th day and died the same day. Monkey 92 treated with fresh pooled immune monkey serum was protected against infection. Monkey 93 treated with the same lot of pooled immune monkey serum which was passed through a filter and preserved with 0.1% tricresol became severely paralyzed and died the 5th day following injection of virus. Monkey 94 treated with immune horse serum developed mild symptoms of infection 12 days after virus injection but was protected against a fatal termination and subsequently made a good recovery.

SERIES 3

All the monkeys (Ring Tails) in this series of experiments received an intravenous injection of 50 c c of a fresh 5% suspension of cord and medulla of a paralyzed monkey as in the experiments recorded in the foregoing.

Exper. 9.—Monkey 95. (Normal horse serum control.) Feb. 19, 1918: Given 2 c c normal horse serum intraspinally.

Feb. 20, 5 p. m.: Intravenous injection of 50 c c of a 5% suspension of virus. Intraspinal injection of 2 c c of normal horse serum.

Feb. 21: Intraspinal injection repeated.

Feb. 22: Given 2 c c normal serum intraspinally.

Feb. 23, 9 a. m.: Flaccid paralysis of both arms and shoulders. Died at 2 p. m.

Necropsy reveals characteristic microscopic lesions of poliomyelitis most marked in the brain stem and cervical cord.

Exper. 10.—Monkey 96. (Immune horse serum.) Feb. 19, 1918: Given intraspinal injection of 2 c c of fresh immune horse serum.

Feb. 20: Intravenous injection of 50 c c of fresh 5% virus suspension. Followed by intraspinal injection of 2 c c of immune horse serum.

Feb. 21: Given 2 c c immune serum intraspinally.

Feb. 22: 2 c c immune serum intraspinally.

Feb. 23: 2 c c immune serum intraspinally.

March 22: Monkey remains well.

Exper. 11.—Monkey 97. (Immune horse serum.) Feb. 19, 1918, 3:30 p. m.: Given an intraspinal injection of 2 c c of fresh immune horse serum.

Feb. 20: Intravenous injection of 50 c c of 5% virus suspension. Monkey received intraspinal injection of 2 c c immune horse serum.

Feb. 21, 22, and 23: Monkey was injected intraspinally with 2 c c of immune horse serum.

March 22: Animal remains well.

Under the conditions of the test the Control Monkey 95 treated by intraspinal injections of normal horse serum became severely paralyzed and died on the 4th day following the intravenous injection of virus. Monkeys 96 and 97 treated with intraspinal injections of immune horse serum remained well.

DISCUSSION

The striking results of the preceding experiments apparently demonstrate that fresh serum from the horse immunized for a considerable period with pure cultures of the coccus, the relation of which to poliomyelitis is under discussion, possesses definite power to prevent poliomyelitis in monkeys injected intravenously with massive doses of virus. Three monkeys were protected perfectly while the 4th monkey developed mild symptoms 14 days subsequent to injec-

tion with virus and recovered completely. The control animals injected with normal horse serum became severely paralyzed in all extremities and died as early as the 4th day after injection with virus.

A far more important point and one which would appear to invalidate the method as a practical test is brought out in the experiments outlined in Series 1. While the monkey receiving immune horse serum was protected perfectly against infection, Monkey 90 treated with fresh immune pooled serum from 2 recovered monkeys became severely paralyzed and died on the 10th day. It appears then that even specific immune monkey serum which is known to possess neutralizing properties for virus may not suffice to prevent infection under the conditions of this test. To overcome this apparent difficulty Amoss and Eberson employed "the mixed serums from several rhesus monkeys which had recovered from experimental poliomyelitis and subsequently received subcutaneous injections of the virus contained in the spinal cord and medulla (reinforced immune)."

Furthermore, the experiments in Series 2 show that while fresh pooled serum from 3 immune convalescent monkeys sufficed to protect Monkey 92 perfectly, this same serum after passage through a Berkefeld filter and the addition of 0.1% tricresol as a preservative acted in the manner of normal horse serum, facilitating a fatal poliomyelitic infection in Monkey 93 5 days subsequent to injection of virus. This apparent discrepancy may be explained by the observation of Flexner and Amoss that foreign serum and very probably tricresol when injected intraspinally act as irritants and set up an aseptic meningitis, thereby facilitating the passage of the virus from the blood into the central nervous system and promoting a poliomyelitic infection.

Accordingly, we have employed only fresh immune horse serum obtained the day of the experiments and used without preservative. It is reasonable to believe that guinea-pig complement and tricresol added to Rosenow's serum as tested by Amoss and Eberson may have produced an aseptic meningitis, thereby promoting a poliomyelitic infection. The accidental puncture of a vein while performing lumbar puncture often occurs and surely would permit the virus to pass directly into the spinal canal in considerable amounts. All of these factors when taken into consideration appear to condemn the method as a practical test of the therapeutic value of serum.

CONCLUSIONS

I have applied the therapeutic test, recently devised by Amoss and Eberson, to fresh samples of immune horse serum prepared by injections of the poliomyelitic coccus in the horse. Under the conditions of the test, 3 monkeys were completely protected while the 4th animal developed mild symptoms on the 14th day and subsequently recovered completely. The control monkeys receiving normal horse serum all developed a fatal poliomyelitis and died as early as the 4th day following injection of virus.

Fresh immune horse serum protected perfectly against infection while pooled immune monkey serum served only to delay the onset of a fatal infection.

The addition of 0.1% tricresol as a preservative to immune monkey serum sufficed to promote a fatal infection in one monkey. The same pooled immune monkey serum when used fresh without preservative protected perfectly against poliomyelitis.

The protective, neutralizing and curative properties of antipoliomyelitic horse serum as reported in previous publications serve to explain the beneficial results previously recorded in the serum treatment of poliomyelitis in man.

The apparent specific neutralizing properties of antipoliomyelitic horse serum for the virus as determined *in vitro* offers a convenient and satisfactory therapeutic test of the serum and seems to add additional proof of the etiologic relationship of the streptococcus to poliomyelitis.